



Docket No.: PA0111
Serial No.: 09/967,301
Inventor(s): Simon Stubbs, et al.
Title: Fluorescent Proteins

Replacement Sheet

Nucleotide Sequence of wtGFP (Chalfie et al, Science, (1994), 263, 802-5):

SEQ ID NO:1

atg agt aaa gga gaa gaa ctt ttc act gga gtt gtc cca att ctt gtt 48
gaa tta gat ggt gat gtt aat ggg cac aaa ttt tct gtc agt gga gag 96
ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa ttt att tgc 144
act act gga aaa cta cct gtt cca tgg cca aca ctt gtc act act ttc 192
tct tat ggt gtt caa tgc ttt tca aga tac cca gat cat atg aaa cgg 240
cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta cag gaa aga 288
act ata ttt ttc aaa gat gac ggg aac tac aag aca cgt gct gaa gtc 336
aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa ggt att 384
gat ttt aaa gaa gat gga aac att ctt gga cac aaa ttg gaa tac aac 432
tat aac tca cac aat gta tac atc atg gca gac aaa caa aag aat gga 480
atc aaa gtt aac ttc aaa att aga cac aac att gaa gat gga agc gtt 528
caa cta gca gac cat tat caa caa aat act cca att ggc gat ggc cct 576
gtc ctt tta cca gac aac cat tac ctg tcc aca caa tct gcc ctt tcg 624
aaa gat ccc aac gaa aag aga gac cac atg gtc ctt ctt gag ttt gta 672
aca gct gct ggg att aca cat ggc atg gat gaa cta tac aaa tag 717

FIGURE 1



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Amino Acid Sequence of wtGFP (Chalfie et al, Science, (1994), 263, 802-5

SEQ ID NO:2

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1 5 10 15
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
20 25 30
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
35 40 45
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
50 55 60
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65 70 75 80
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
85 90 95
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100 105 110
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
130 135 140
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145 150 155 160
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
165 170 175
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

FIGURE 2



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Predicted Amino Acid Sequence of F64L-S175G-E222G-GFP:

SEQ ID NO:3

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1 5 10 15
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
20 25 30
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
35 40 45
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
50 55 60
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65 70 75 80
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
85 90 95
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100 105 110
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
130 135 140
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145 150 155 160
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val
165 170 175
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Gly Phe Val
210 215 220
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

FIGURE 3



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Replacement Sheet

Predicted Amino Acid Sequence of F64L-S65T-S175G-GFP:

SEQ ID NO:4

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1 5 10 15
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
20 25 30
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
35 40 45
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
50 55 60
Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65 70 75 80
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
85 90 95
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100 105 110
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
130 135 140
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145 150 155 160
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val
165 170 175
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

FIGURE 4



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Flow cytometry of GFP mutations

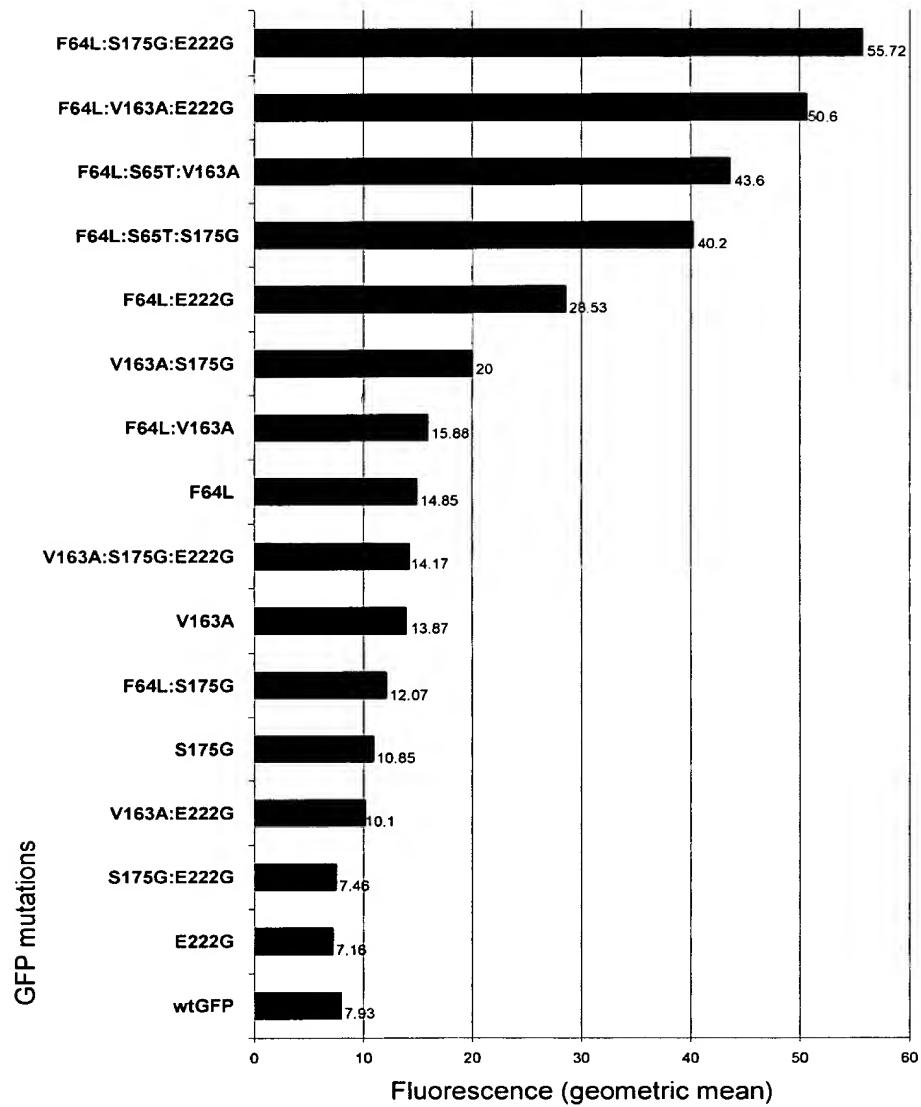


FIGURE 5



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Photobleaching of GFP mutations

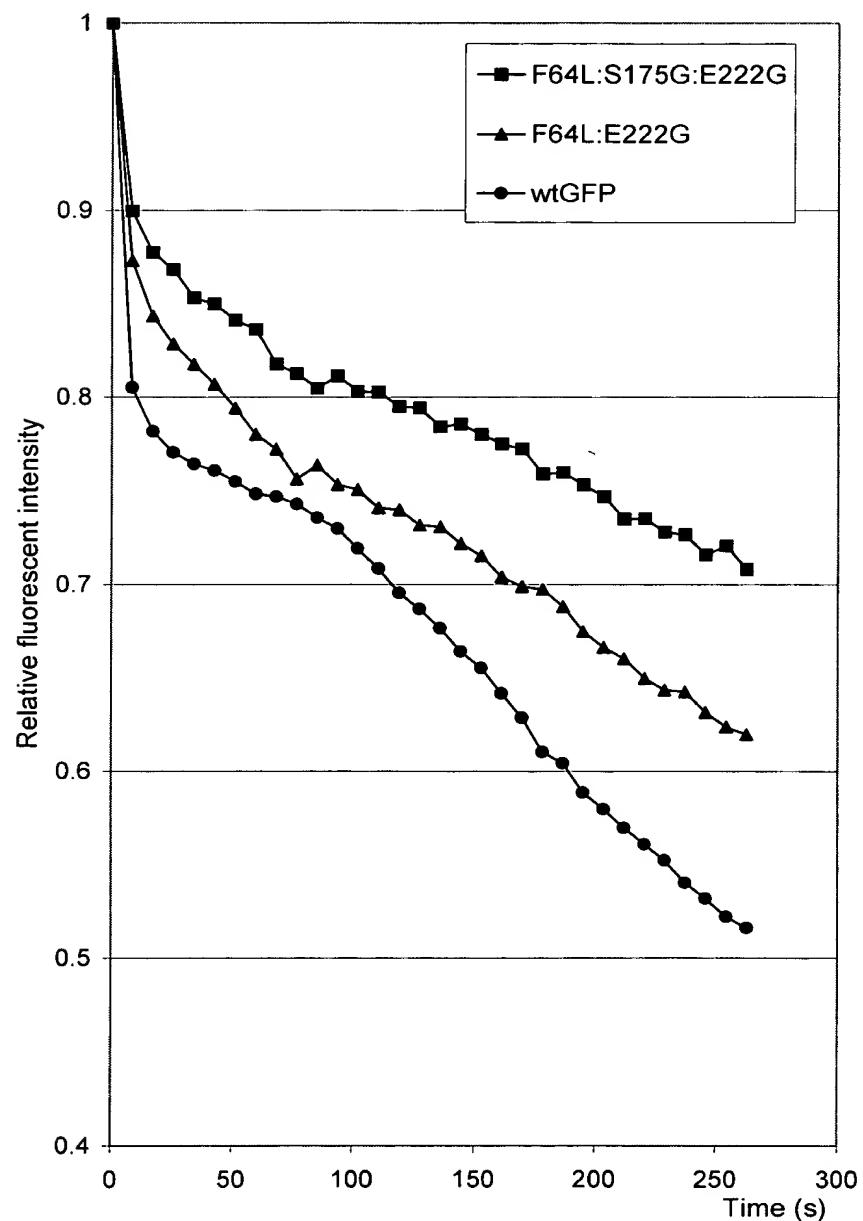
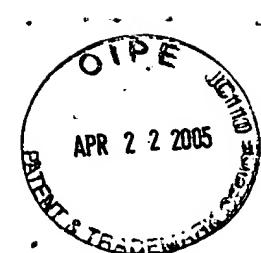


FIGURE 6



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Live Cell CHO-HIR NF κ B Assay
P65-tri GFP Assay T = 30 mins

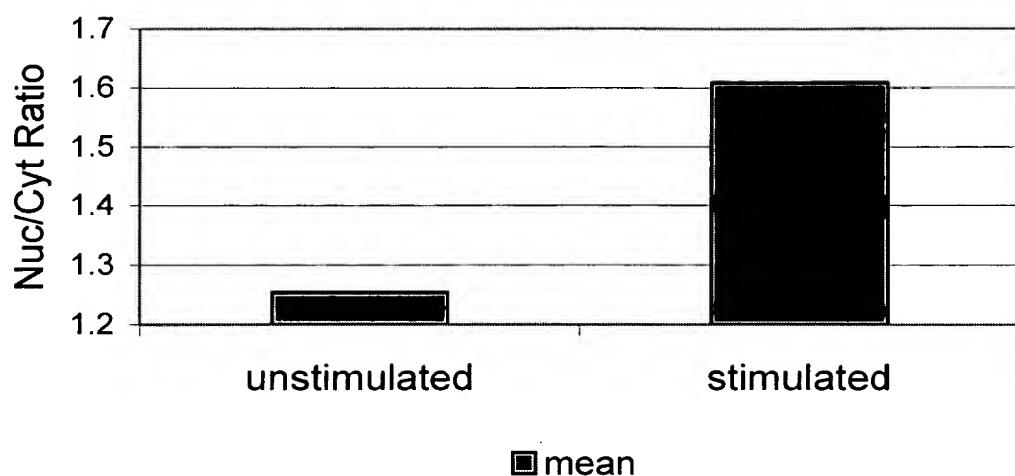


FIGURE 7